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Disease Progression in Myelodysplastic Syndromes: Do Mesenchymal Cells Pave the Way?

Marc H.G.P. Raaijmakers[1,*]
[1]Department of Hematology, Erasmus MC Cancer Institute, and Erasmus Stem Cell Institute, Faculty Building Rm Ee-1393, dr. Molewaterplein 50, 3015GE, Rotterdam, The Netherlands
*Correspondence: m.h.g.raaijmakers@erasmusmc.nl
http://dx.doi.org/10.1016/j.stem.2014.05.010

Early events driving the initiation and evolution of neoplasms remain poorly defined but involvement of an instructive or permissive niche has been implicated. In this issue of *Cell Stem Cell*, Medyouf et al. (2014) provide insights into the role of the niche in myelodysplastic syndromes, the principle preleukemic disorder of the hematopoietic system.

Myelodysplastic syndrome (MDS) is considered a clonal disorder of hematopoietic cells characterized by bone marrow failure and predisposition for evolution into acute myeloid leukemia (AML). The etiology of the disease, including the cell of origin and the factors driving leukemic progression, remains poorly understood. Progress in this area has been hampered by the inability to faithfully engraft and propagate human disease in murine xenograft models, an observation that has sparked a longstanding debate about a potential causative or facilitating role of the microenvironment in the pathogenesis of this disease. Medyouf et al., in a series of challenging experiments, now implicate mesenchymal cells in disease initiation and progression, thus creating a humanized model of MDS (Medyouf et al., 2014).

In a cohort of genetically heterogeneous, lower risk MDS patients, the authors demonstrate that coinjection of $CD34^+$ hematopoietic cells, together with their corresponding in vitro expanded mesenchymal stromal cells (MCs), into the marrow cavity of NOD/LtSzscid-IL2rg$^{-/-}$ (NSG) mice significantly increases reconstitution with human MDS cells in comparison to transplantation with $CD34^+$ alone or coinjection with MCs from age-matched normal bone marrow.

Repopulating cells were bona fide MDS cells, as shown by lineage and clonality tracking, and included a cell type with long-term repopulation ability. MDS cells with reconstitution ability (''disease-propagating cells'') in this experimental setting were found exclusively within the $CD34^+lin^-CD38^-$ subset (albeit tested in a limited number of patients), which, together with the observation that mutations occur in multiple lineages in MDS patients, suggests that initiating mutations in this disease occur in primitive multipotent hematopoietic cells, at least in a subset of patients.

Transcriptional profiling aimed at interrogating the signaling mechanisms underlying the disease propagating effects of MCs revealed aberrant gene expression in MDS-MCs, including gene sets implicated in intercellular crosstalk, osteo/adipogenesis, inflammation, and fibrosis, which are all processes related to clinico-pathologic characteristics of subsets of human MDS. MDS hematopoietic cells were shown to have the capacity to induce similar transcriptional changes in MCs in coculture experiments.

Together, the findings make an important contribution to the field by establishing a more robust in vivo method to model human MDS. They further provide experimental support for the view that in MDS, reciprocal heterotypic signaling between disease-propagating hematopoietic cells (and/or their progeny) and mesenchymal elements within the bone marrow environment is required to drive disease initiation and progression, at least in the context of the xenograft model. Such reciprocal signaling may be initiated through ''reprogramming'' of mesenchymal elements by hematopoietic cells and subsequently lead to a cascade of yet-to-be-defined events facilitating disease progression.

While the findings clearly establish a functional role for MDS-MCs in the context of the xenograft model, translation of this finding to its significance for human disease biology is not entirely straightforward. First, the cellular, molecular, and functional relationships between ex vivo expanded MCs and their in situ niche equivalents remain to be fully defined. Second, transplanted MCs were detected only in the injected femur and disappeared within several weeks, while long-term engraftment and expansion of MDS cells was observed in both femurs, indicating that MCs facilitated initial homing and engraftment, but were not required for long-term maintenance of MDS cells or seeding to the contralateral femur. While it is tempting to hypothesize that human MDS cells, after initial engraftment, may be able to establish their own self-enforcing murine niche, this remains to be shown. Future studies, aimed at elucidating the underlying molecular mechanisms of MC-facilitated engraftment of MDS cells, including the demonstration that this process encompasses MC-hematopoietic cell interactions, will shed further light on these open questions.

Nevertheless, the differential ability of MDS-MCs (in comparison to normal MCs) to promote MDS engraftment, and the heterotypic induction of transcriptional programs in MDS-MCs implicated in disease biology, strongly argue in favor of a view in which mesenchymal elements contribute to disease pathogenesis in MDS. This notion seems congruent with emerging experimental insights in support of a critical role of the mesenchyme in disease initiation and progression, both in MDS and other hematopoietic neoplasms (Walkley et al., 2007, Raaijmakers et al., 2010, Schepers et al., 2013, Kode et al., 2014, Scadden, 2012).

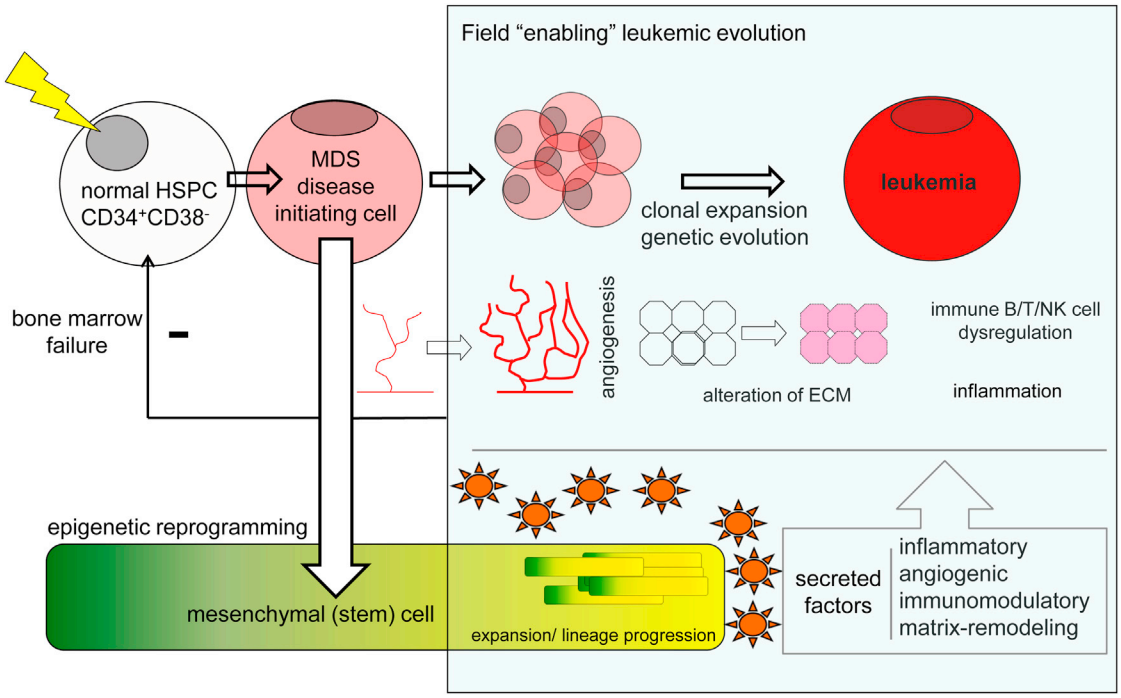

Figure 1. A Hypothetical Model of Heterotypic Reciprocal Signaling Driving Disease Progression in MDS
Early genetic events in a primitive hematopoietic cell generate "MDS disease initiating cells" that instruct epigenetic reprogramming of mesenchymal (stem) cells. These reprogrammed mesenchymal cells expand and create a mutagenic field "enabling" clonal expansion and genetic evolution of disease-initiating cells at the cost of normal hematopoiesis.

It therefore seems reasonable to ask the question of how, in principle, reprogramming of mesenchymal cells could contribute to disease pathogenesis in MDS within the framework of our current knowledge of their biology (Morrison and Scadden, 2014, Frenette et al., 2013) and the "enabling" processes underlying neoplastic initiation and progression in other systems (Hanahan and Weinberg, 2011).

Mesenchymal elements in the bone marrow constitute a heterogeneous population of functionally distinct cells that may descend from primitive (stem) cells in a linear hierarchy. They play critical roles in the maintenance of tissue composition and normal HSC and progenitor subsets (including B cell progenitors) (HSPCs) and exert immuno-regulatory functions in adaptive and innate immunity (i.e. NK, dendritic, and T cells). Heterotypic signaling from diseased "reprogrammed" mesenchymal (stem) cells may thus affect other cell types and processes within the complex cellular circuitry of the bone marrow, promoting angiogenesis, remodeling of the extracellular matrix (fibrosis), and attenuation of normal HSPC function, facilitation of mutagenesis and escape from immunosurveillance for evolving neoplastic clones. Indeed, these processes reflect clinical MDS features and gene signatures in MDS-MCs described by Medyouf et al., including upregulation of angiogenic, matrix-modulating and inflammatory pathways. This understanding enables a view in which reciprocal signaling between mesenchymal cells and other components of the niche, perhaps through a series of (forward-feeding) events, critically contributes to a permissive or mutagenic environment, enabling leukemic evolution of MDS cells, not unlike the establishment of premetastatic niches in epithelial tumor biology (Oskarsson et al., 2014) (Figure 1).

Deciphering the molecular mechanisms driving MC reprogramming and heterotypic signaling in MDS will provide key insights to test this hypothetical view. Of note, the transcriptional profiling of MDS-MCs conducted by Medyouf et al. identified molecular commonalities between patients, despite genetic disease heterogeneity, which highlight common triggers that may be amenable to therapeutic targeting. Of interest, "reprogramming" may occur through epigenetic alterations, a view supported by the observation in this study that a hypoxia gene signature was maintained under normoxic culture conditions, but also supported by longstanding observations that MDS-MCs display aberrant characteristics after extensive serial passaging ex vivo. Epigenetic changes could help explain how alterations in a single mesenchymal (stem) cell in early phases of disease could be transferred into distinct subtypes of mesenchymal cells upon lineage progression or to a larger number of cells through proliferative expansion, thus creating a "field" of ancillary cells contributing to malignant progression. It may also contribute to our understanding about the therapeutic efficacy of hypomethylating therapy, including its potential to delay leukemic progression.

In conclusion, the study of Medyouf et al. (2014) marks an important step toward modeling human MDS. It also underlines the importance of approaching preleukemic disorders from a systems biology point of view, helping us appreciate the intricate circuitry of heterotypic

cell-cell communication to increase our fundamental understanding of how pre-malignant cells utilize the microenvironment to promote their malignant agenda. These insights will ultimately result in novel therapeutic strategies, targeting niche cells to attenuate leukemic progression.

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Common Ground: Stem Cell Approaches Find Shared Pathways Underlying ALS

Soledad Matus,[1,2,*] Danilo B. Medinas,[1,3] and Claudio Hetz[1,2,3,*]
[1]Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Independencia 1027, Chile
[2]Neurounion Biomedical Foundation, Santiago, Chile
[3]Program of Cellular and Molecular Biology, Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Independencia 1027, Santiago, Chile
*Correspondence: soledad.matus@neurounion.com (S.M.), chetz@med.uchile.cl (C.H.)
http://dx.doi.org/10.1016/j.stem.2014.05.001

The development of curative therapies for genetically complex diseases such as ALS has been delayed by the lack of relevant disease models. Recent advances using induced-pluripotent-stem-cell-derived motoneurons from patients harboring distinct ALS mutations have recapitulated essential disease features and have identified some common pathways driving disease pathogenesis.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder involving the loss of motoneurons in the spinal cord and motor cortex, leading to paralysis and premature death. Whereas most ALS cases are sporadic (sALS), around 10% are considered familial and mainly inherited in a dominant manner (fALS), involving mutations in more than two dozen genes, including superoxide dismutase 1 (SOD1), Tar DNA-binding protein-43 (TDP-43), fused-in-sarcoma (FUS), and *C9orf72*. The biology of ALS is very complex; however, it is known that the pathology is specific to motor neurons. Multiple pathogenic mechanisms have been proposed to contribute to the selective motoneuron degeneration, including alterations in RNA metabolism, mitochondrial dysfunction, abnormal protein aggregation, endoplasmic reticulum (ER) stress, excitotoxicity, axonal transport defects, and gliosis, among other events.

Most cellular and animal models of ALS involve the expression of supraphysiological levels of human transgenes, mostly involving mutant SOD1 and TDP-43, which contrasts with the single allele alteration observed in fALS cases. Although these ALS models have been fundamental to investigate disease pathogenesis, drugs identified to abrogate or delay experimental ALS using these models have failed in clinical trials. These shortcomings underscore the need for novel model systems to assess unique events that may underlie the selective neuronal vulnerability observed in human cells. Induced pluripotent stem cells (iPSCs) generated from ALS patients and differentiated into motoneurons represent a promising new tool for studying ALS disease pathology. Using stem cell approaches, three recent studies reported in *Cell Stem Cell* (Kiskinis et al., 2014; Chen et al., 2014) and *Cell Reports* (Wainger et al., 2014) revealed the occurrence of inherited abnormal cellular phenotypes in ALS patient-derived motoneurons involving distinct genetic mutations. Remarkably, common pathways of degeneration have emerged through these studies (Figure 1), opening the possibility of new and effective drug screening.

Kiskinis et al. (2014) and Chen et al. (2014) both established new in vitro models of ALS by generating iPSC-derived motoneurons from patients carrying SOD1 mutations. Both studies developed a well-controlled cellular system by combining genetic correction of the SOD1 mutation and whole-genome